Fast and efficient HLA typing analysis using D1K ScreenTape

Application Note

Nucleic Acid Analysis

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Abstract

The Agilent 2200 TapeStation system enables simple and convenient analysis of PCR sequence specific primer (SSP) amplicons for human leukocyte antigen (HLA) genotyping. Allele-specific PCR combined with automated electrophoresis rapidly enables detection, interpretation, and storage of tissue typing results.

Introduction

HLA genes, divided into class I and class II, code for cell surface molecules that play an essential function in the immune response by allowing the body to discriminate between self antigens and non-self antigens, typically pathogens. HLA typing is important for organ and bone marrow transplants, in vaccine trials, and as an aid to disease diagnosis. The HLA region is highly polymorphic; therefore, matching specific HLA alleles is necessary to improve the transplantation success rate between donor and recipient.

Experimental

Material

DynalAllSet and SSP DQB1 low resolution kit (batch EK0582) was purchased from Invitrogen Life Technologies (Carlsbad, CA, USA); 2200 TapeStation System, D1K ScreenTape, and D1K Reagents were obtained from Agilent Technologies (Waldbronn, Germany).

PCR assay

Genomic DNA samples were amplified using HLA typing set according to the manufacturer's recommendations. Amplification time was 80 minutes.

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D1K ScreenTape analysis procedure

For a single genotype, seven allele-specific SSP amplicons from one sample were mixed 1:3 with D1K sample buffer and placed in the 2200 TapeStation instrument along with a D1K ScreenTape. The analysis was started from the 2200 TapeStation software; the amplicons were separated, imaged, and sized within 10 minutes.

Results and Discussion

The gel image of allelic DQB1 genotyping by PCR-SSP technique is shown in Figure 1. The 2200 TapeStation software displays the results automatically as a gel image, as electropherograms, and in a tabular format containing fragment sizes in bp. This simplifies the determination of positive or negative PCR reactions. The D1K sample buffer contains lower and upper markers at 25 bp and 1500 bp for accurate sizing. Each PCR-SSP reaction contains a ~ 607 bp control amplicon shown in all reactions (lanes 2-7). Lanes 2 and 3 show specific bands at ~ 210 bp that correlate to a DQB1*05 and DQB1*06 genotype.

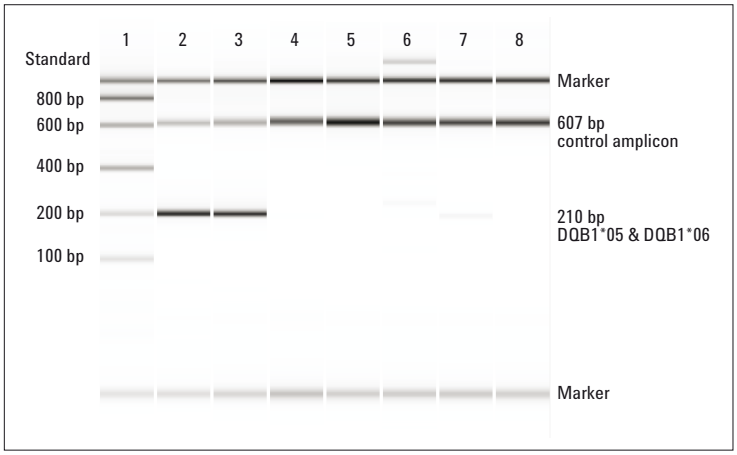

Figure 1
2200 TapeStation gel image of a DQB1 low resolution specific PCR.

Conclusion

The Agilent 2200 TapeStation system enables swift identification of numerous variant alleles at HLA loci, by analyzing locus specific PCR products quickly and efficiently. Pre-packaged reagents and full automation result in highly reproducible data with minimal user intervention. Intuitive software streamlines the laboratory workflow and automatic archiving of results delivers convenient traceability for important data.

www.agilent.com/genomics/tapestation

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